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United States Department of Agriculture — Agricultural Research Service research on alternatives to methyl bromide: pre-plant and post-harvest†‡

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Abstract: Methyl bromide is a widely used fumigant for both pre-plant and post-harvest pest and pathogen control. The Montreal Protocol and the US Clean Air Act mandate a phase-out of the import and manufacture of methyl bromide, beginning in 2001 and culminating with a complete ban, except for quarantine and certain pre-shipment uses and exempted critical uses, in January 2005. In 1995, ARS built on its existing programs in soil-borne plant pathology and post-harvest entomology and plant pathology to initiate a national research program to develop alternatives to methyl bromide. The focus has been on strawberry, pepper, tomato, perennial and nursery cropping systems for pre-plant methyl bromide use and fresh and durable commodities for post-harvest use. Recently the program has been expanded to include research on alternatives for the ornamental and cut flower cropping systems. An overview of the national research program is presented. Results from four specific research trials are presented, ranging from organic to conventional systems. Good progress on short-term alternatives is being made. These will be used as the foundation of integrated management systems which begin with pre-plant management decisions and continue through post-harvest processing.
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1 INTRODUCTION

Methyl bromide is a critical element in pre-plant management of soil-borne pests and pathogens in high value fruits, nuts, vegetables, nursery and ornamental crops, and in post-harvest management of pests and pathogens on fresh produce and durable commodities. The Montreal Protocol, an international treaty, and the US Clean Air Act restricted availability of methyl bromide; beginning in January 2001, its use was restricted to 50% of the amount used in the baseline year of 1991, further restricted to 30% of the baseline in 2003 and completely banned in 2005. Quarantine use of methyl bromide is exempted from the impending ban. US growers of high value crops are in dire need of alternative management strategies for both pre-plant and post-harvest uses. The availability of acceptable alternatives will impact upon the supply and quality of these foods to both American consumers and the export market. In response to the urgent need, the United States Department of Agriculture—Agricultural Research Service (ARS) initiated a diversified research program in 1995 that spans 10 states and Washington, DC, expenditure for which has grown to approximately $15 million in 2001. This paper overviews the research program and presents a sampling of research highlights.

2 PRE-PLANT

Pre-plant soil fumigation with methyl bromide is used to control ‘replant disorder’, weeds, and soil-borne pathogens for many high value crops including strawberries, tomatoes, peppers, ornamentals, nursery crops, grapes, tree fruit and nut trees. The phasing out

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of methyl bromide raises two major issues. The first is to quickly find effective, alternative control measures. Methyl bromide can be used effectively against a broad spectrum of soil pests over a range of soil types, temperature and moistures resulting in greater flexibility and less risk of loss than is possible with other soil treatments. Unless a 'silver bullet' that is effective against a wide range of pests can be found, the first challenge will be to accurately diagnose the problem(s) in a specific field. Once the problem is identified, a management strategy must be generated that is (1) effective against the identified pest, (2) effective under the soil conditions found in that field, (3) economically feasible, and (4) environmentally acceptable. ARS is addressing this issue by testing plant growth promoting rhizobacteria (PGPR), suppressive soils, soil amendments, fallow, mulches, crop rotation, host resistance, new chemicals and new application technologies to deliver biological and chemical alternatives. The second issue is to increase understanding of the pathogens and soil factors limiting crop production. A long-term, integrated management approach requires a thorough understanding of biological, chemical and physical soil factors, their interactions and their spatial variability, and will include cultural, genetic, biological and chemical management strategies. The short-term solutions to methyl bromide alternatives are stepping stones to the long-term research into integrated systems.

2.1 Annual crops

Annual crops have traditionally relied on methyl bromide treatment before each cropping season to control soil-borne pathogens, nematodes and weeds. The level of pest and pathogen control achieved with methyl bromide in annual crops is often the cumulative result of annual fumigation over many years. As methyl bromide is phased out, unexpected pests and pathogens that were unknowingly controlled by the annual methyl bromide fumigation are likely to appear. ARS research has focused on strawberry and vegetable production systems in California and Florida. Recently, ARS created two new positions to address the needs of flower and ornamental production systems.

2.1.1 Florida tomato and pepper production

2.1.1.1 Overview

Florida is the leading producer of fresh market tomato and bell pepper in the USA, with 23 760 ha producing a crop valued at US $745 million.[1] The fresh market tomato industry in Florida accounts for more than one-third of the US crop[2] and use of methyl bromide has been estimated to be as high as 94% of the acreage planted to tomatoes annually.[3] Root-knot nematode, fungal plant pathogens and weeds are of great concern in the absence of methyl bromide. Together, the Florida tomato and pepper industries account for 8% of the global consumption of methyl bromide[4] and, without viable alternatives, production is predicted to decline by 60% and 63% respectively.[5] In Florida, growers use a 'raised-bed plastic mulch' production system that consists of seedlings transplanted into preformed beds that have been fumigated with methyl bromide and covered with polyethylene plastic.[6,7] Although there are registered soil fumigants that show promise for disease and nematode control, there are limited numbers of effective herbicide partners available for these crops.

2.1.1.2 Cultural and genetic control

Preliminary studies on the use of paper mulch for nutsedge control in the raised-bed vegetable production system have shown that paper provides excellent weed suppression that is equivalent to or better than the use of the combination of 1,3-dichloropropene (1,3-D; Telone) + chloropicrin and pebulate (Tillam) (Rosskopf EN, unpublished). Nematode-susceptible bell pepper cultivars grown in fields previously cropped with the resistant cultivar Carolina Cayenne[8] had reduced galling from the root-knot nematode, *Meloidogyne incognita* (Kof & White) Chitwood, and yield was 2.8 times greater than in plots previously cropped to a susceptible variety.[9]

2.1.1.3 Biological control

Research on the use of plant-growth-promoting-rhizobacteria (PGPR) in combination with soil disinfestation treatments contributed to the development of BioYield™. The combination of bacterial strains LS213 and LS256 with methyl bromide resulted in higher pepper yields than with methyl bromide alone.[10] Fungal plant pathogens, developed as components of an integrated approach to weed management, are highly host specific and affect only the target weed.[11] *Dactylaria higginsii* (Lutrell) MB Ellis is being tested currently as an off-season treatment for control of nutsedge and as a post-emergence spray in combination with 1,3-D + chloropicrin for tomato production.[12] *Phomopsis amaranthicola* Rosskopf, Charud., Shabana & Benny, a pigweed pathogen, is an excellent candidate for use in the ornamentals production system, where few herbicides are available.[13] Non-pathogenic strains of *Fusarium oxysporum* Schlecht, isolated from tomato roots grown in a suppressive soil, provide significant and consistent control of Fusarium wilt of tomato.[14,15]

2.1.1.4 Chemical control

On-going cooperative research projects with scientists at the University of Florida include various application techniques with existing chemical alternatives[16] as well as the development of new chemistries, such as propargyl bromide[17] and reduced-risk compounds.[18] Several years of field trials have been conducted with Plant Pro45™, an iodine-based material, generating encouraging results in the control of root-knot nematode, the fungus *Fusarium oxysporum* f sp *basili* Tamietti & Matta, and several weed species.[19–21]

2.2 Research highlight: large scale field trials

Large-scale field trials were instrumental in identifying technical problems not evident in small-scale research plots, developing information on control of soil-borne pests under the range of environmental and cultural practices experienced by growers, generating information on costs incurred at the farm level, and providing growers with the experience to evaluate alternatives.

2.2.1 *Soil solarization field trial*

Initially, soil solarization was evaluated in Florida as a broadcast treatment and found to control several key soilborne pests.[22–24] It was not compatible with local production systems, however, due to the increased cost of the plastic and the accumulation of storm water run-off during heavy rains. Soil solarization was adapted to local production systems by performing strip solarization with clear plastic on the same beds used for production. Solarization on raised beds produced higher soil temperatures than broadcast solarization on a flat surface, eliminating the border effect associated with soil solarization and improving its efficacy.[25] Painting the clear plastic with white paint allowed it to function as horticultural mulch and terminated the solarization period. In research trials, significant control of most soil-borne pests was obtained, the exception being rootknot nematode.[25–27]

Twenty-one large-scale field trials of soil solarization have been conducted on a total of 44.1 ha over the years 1995–1999. To address the lack of control of root-knot nematode, some trials included fumigation with 1,3-D + chloropicrin and use of disease-resistant cultivars. Pest control and marketable yield were measured and compared with data obtained from adjacent methyl bromide treated areas. Weed control in solarized plots was comparable with that in adjacent methyl bromide plots in all locations except when purslane (*Portulaca oleracea* L) and Texas panicum (*Panicum texanum* Buckl) were present. Root gall ratings indicated that soil solarization did not provide adequate control of root-knot nematodes. When solarization was combined with reduced rates of 1,3-dichloropropene + chloropicrin, reductions in root galling were similar to those achieved in adjacent methyl bromide fumigated plots. The incidence of soilborne diseases caused by *Fusarium oxysporum* f sp *lycopersici* Snyd & Hans, *Phytophthora capsici* Leonian and *Sclerotium rolfsii* Sacc were similar in solarized and methyl bromide treated plots in all trials except the 1999 tomato trial, where the incidence of Fusarium wilt was significantly higher in solarized beds. In the tomato field trials, marketable yields in solarized plots were between 85% and 106% of yields obtained in adjacent methyl bromide treated plots (Table 1). Soil solarization resulted in an average reduction in yield of 5% (Table 1). Fumigation with mixtures of 1,3-D and chloropicrin at the initiation of solarization did not improve yields, even though the control of nematodes was improved (Table 1). Large-scale field trials identified several technical problems not evident in smaller research plots. When using drip irrigation, the tube must be covered with soil during the solarization period to prevent melting. Paint coverage at the termination of the solarization period must be uniform and complete to prevent any additional solar radiation from penetrating the plastic and heating the soil to levels detrimental to the health of the transplants.

2.2.2 *Broadcast fumigation field trial*

When combined with the herbicide pebulate, shank injection of 1,3-D plus chloropicrin (Telone C17 and Telone C35) into beds prior to laying plastic has provided pest control and yields similar to those achieved with methyl bromide.[28,29] Application of 1,3-D requires personal protective equipment (PPE) to be worn by workers during application.[30] If 1,3-D were applied via shank injection during bedding, which is the recommended procedure for methyl

Table 1. Marketable yields obtained from large-scale trials using soil solarization

Farm	Year	Crop	Treatment	Marketable yield (kg ha^{-1})	Comparison with methyl bromide (%)
1	1995	Tomato	Solarization	60 536	98
2	1995	Tomato	Solarization	54 320	106
3	1995	Tomato	Solarization	44 324	85
4	1996	Tomato	Solarization	48 244	95
5	1997	Tomato	Solarization	55 328	92
6	1998	Tomato	Solarization	36 512	96
Average				54 430	95
1	1995	Tomato	Solarization + 1,3-D:chloropicrin (83:17 mixture at 164 liter ha^{-1})	48 870	88
2	1996	Tomato	Solarization + 1,3-D:chloropicrin (83:17 mixture at 164 liter ha^{-1})	43 980	81
3	1996	Tomato	Solarization + 1,3-D (93 liter ha^{-1})	67 620	92
4	1999	Tomato	Solarization + 1,3-D (112 liter ha^{-1})	39 090	79
5	1999	Tomato	Solarization + 1,3-D:chloropicrin (65:35 at 327 liter ha^{-1})	45 030	94
Average				48 930	87

bromide application in Florida vegetable production, 30–50 workers would have to wear full protective suits and charcoal respirators. This is impractical and potentially dangerous in high summer temperatures. A deep placement coulter system (Avenger, Yetter Manufacturing Co, Colchester, IL) was modified to permit injection of 1,3-D into undisturbed soil at 30 cm depths and seal the soil above the injection point without creating channels for the fumigant to escape (John Mirusso, Mirusso Fumigation and Equipment, Delray Beach, FL). Ten large-scale field trials on a total of 31.5 ha were conducted using this technology during 2000–2001. Herbicide applications were combined with different formulations and rates of 1,3-D + chloropicrin and applied using the deep placement coulter system. In some trials, chloropicrin was shank injected into beds prior to laying plastic. In all ten trials, no difference in the density of plant parasitic nematodes was observed between experimental treatments and adjacent methyl bromide fields. Similar levels of weed control were observed in eight of ten trials, but the chemical alternatives failed to provide weed control in two trials conducted on farms with high populations of nightshade (*Solanum nigrum* L). Lack of control was attributed to grower inexperience with broadcast applications of pre-emergence herbicides. In the field trials where fumigant applications were made between November and February, levels of disease control similar to those with methyl bromide were achieved with a broadcast application of 1,3-D and chloropicrin. When fumigant applications were made between July and September, an additional application of chloropicrin in the bed was required to achieve levels of disease control similar to those with fumigation with methyl bromide, due to the increased disease pressure during the warmer crop production months.

Application methods and technology were modified to make soil solarization and broadcast fumigation compatible with Florida crop production systems. In both cases, adaptation to local production systems enhanced their performance. When used as a single tactic, soil solarization and 1,3-D + chloropicrin did not provide the same broad level of pest control as methyl bromide. A comprehensive pest management program that included additional tactics such as herbicides, fumigants and/or resistant cultivars was required to provide pest control similar to methyl bromide on a consistent basis.

3 CALIFORNIA STRAWBERRY PRODUCTION

3.1 Overview

California has the most productive strawberry fields in the world due to 50 years of research on optimizing cultivars and cropping practices in the context of soil fumigation with methyl bromide + chloropicrin (MBC). The production system is also based on vigorous, clean transplants, which are grown in MBC-fumigated nursery soils. In fruiting and nursery fields, fumigation with MBC is essential for weed, disease and nematode control, and non-specific growth promotion. In fruiting fields, fumigation insures a return on the investment required for crop establishment (~\$25 000 ha^{-1}) and total crop production (~\$62 000 ha^{-1}).

ARS research has focused on alternatives that can be implemented immediately with little change in cultural practices. Large multidisciplinary projects test currently available chemicals in fruiting and nursery fields as one-for-one replacements.[31,32] Tests of alternative chemicals, application technologies and the use of alternative tarps for fumigation have provided growers with hope of short-term sustainability.[31,33–35] Movement and distribution of the fumigants are being evaluated to optimize the use of new chemicals.[36,37] Plant pathologists have used many of the same chemical trials to document the influence of alternative chemicals on specific plant pathogens[38,39] and potentially beneficial microorganisms.[39,40] Understanding the ecology of these organisms is important for controlling plant diseases in the absence of fumigation.

To avoid potential regulatory restrictions on chemicals under evaluation, non-chemical alternatives to chemical fumigants are being investigated. Rotations with broccoli reduced inoculum of the most important soil-borne pathogen of strawberry, *Verticillium dahliae* Kleb, and increased yield.[41] Microbial inoculants are being evaluated and developed for soilborne diseases[39,40,42] and will be used in conjunction with other management strategies for control of weeds.

ARS has taken the lead on a multi-agency and multidisciplinary (entomology, weed science, plant pathology and sociology) project, which enlists growers to help design, improve and implement a biologically integrated production system for strawberries. The new practices provided plant growth stimulation and weed control (Reference 43 and Fennimore S, pers comm) but improvement in the system is needed before adoption will occur.

3.2 Research highlight: organic strawberry production

ARS is also working to improve alternative cropping systems that are already in place. Despite the high risk involved in strawberry production in the absence of methyl bromide, the acreage of certified organic strawberry production has increased. The number of organic strawberry growers certified by a leading certification organization in California has increased by 27% from 34 to 46 from 1999 to 2001.[44,45] Experiments comparing organic and conventional production have reported yields for the organic plots as high as 72% of conventional yields.[46] That growers are able to attain these yields, often on marginal land and with virtually no research support, suggests that research conducted in an organic context should help to optimize their production systems.

Although choice of variety is very important for success, the performance of commercially available cultivars had never been compared in organic fields. High yielding cultivars were evaluated and selected for their yield and fruit quality in conventional production practices.[47] Organic growers rely on cultivars designed for use in MBC-fumigated soils, although cultivars perform differently in fumigated and non-fumigated conventional fields.[48] Because, in addition to the lack of fumigation, organic producers do not use fungicides or pesticides, it is likely that these differences in performance will be exaggerated in organic fields. This makes cultivar camparisons in conventional fields less relevant for cultivar selection for organic production.

A comparison of commercially available cultivars in organic production fields was completed by ARS scientists and collaborators. Experiments compared 15 cultivars at three locations during two field seasons in the central coast region of California. Overall, the cultivars Aromas, Pacific and Seascape performed the best in these trials.[49] Aromas, Seascape and Pacific have each been shown to be tolerant to at least one of the following soilborne pathogens: *Pythium ultimum* Trow, *Phytophthora cactorum* Schroet and *Verticillium dahliae* Kleb.[38,39,50]

Planting material is about to become a more significant problem for organic growers. The USDA's new National Organic Standards will require that growers obtain a quality of transplant that is currently not available. Currently organic growers use conventionally grown strawberry transplants as a default because organically grown transplants are not available. Disease-free organic transplants can be produced under greenhouse conditions. In an organic production field, the performance of organic plug plants was compared with conventional field transplants. The experiment was a randomized complete block experiment with four replications. Berries were harvested once a week with cull and market quality fruit weighed separately. Data for yield are the cumulative data for all harvests. Conventional transplants were planted as recommended for adequate chilling. Plug plants were planted on the same day as bare-root transplants. There were significant differences between conventionally produced plants and organic plug plants for market ($P = 0.002$) and total ($P = 0.0009$) yield. Plug plants had significantly lower yields than the conventional transplants (Fig 1). Additional research is needed to determine optimum planting dates and conditions for production from organic plug plants. It will take time to optimize any new production system to achieve yields comparable with the current system, which has benefited from five decades of farmer innovation and scientific research.

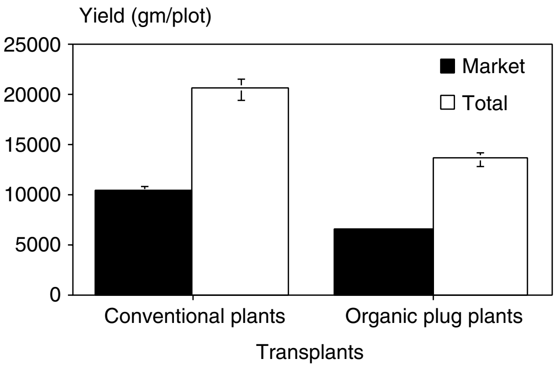

Figure 1. Market and total yield of conventional transplants and organic plug plants grown in a certified organic strawberry production field. Perpendicular lines at the top of the bars represent the standard error of the mean.

4 FLORICULTURE AND ORNAMENTALS

The floriculture and ornamental industry is highly diverse and includes the production of ornamental nursery plants, potted plants, cut flowers, foliage and bulb crops. The California floriculture industry had a wholesale value of $842 million in 2000.[51] The cut flower industry in California was worth approximately $286 million.[51] In Florida, the value of this commodity is approximately $29 million.[52] Florida produces approximately 95% of the world's caladiums (*Caladium* spp),[53] which had a value of $15 million last year (Terri Cantwell, Bates Sons and Daughters, pers comm). The diversity of crops that are grown in the floriculture and ornamentals nurseries in California and Florida represents a very complex research problem. While the total methyl bromide consumption by these commodities is difficult to estimate, the loss of this fumigant will present a unique challenge to these growers. The ornamentals industry is composed of hundreds of species of crops and thousands of varieties. Due to the number of different crops that are grown concurrently and in succession, the issue of phytotoxicity of alternative chemicals is extremely important. In addition to having severe limitations on the number of registered pesticides that are labeled for these crops, there is a requirement for clean propagation material in order for it to be shipped between states and to other countries. Weed control with a limited number of labeled herbicides and the need for clean, certifiable stock are the greatest concerns for growers of these commodities.

USDA research in this area is relatively new, although there are many technologies that have been developed for other crops that may have application for floriculture and ornamental production. Soil solarization has potential for these crops and some pathogens that have been found to be problems in ornamentals can be controlled in this manner.[54] Botanical extracts and essential oils have also been investigated as alternatives to methyl bromide for control of Fusarium wilt caused by *Fusarium oxysporum* Schlecht f sp *chrysanthemi* Luttr, GM Armstrong & JK Armstrong. Soil infested with this pathogen was treated with aqueous emulsions of formulated extracts of clove, neem, chili pepper extract and essential oil of mustard, and cassia. Ten per cent solutions of pepper/mustard, cassia, and clove extracts significantly reduced the soil populations of the pathogen.[55] These extracts could be used as a component in a biologically based integrated pest management strategy for ornamentals.

5 PERENNIAL CROPS

Pre-plant methyl bromide use in orchards and vineyards in the USA totaled 2700 metric tons in 1997, accounting for approximately 15% of the total pre-plant use in the USA.[56] Perennial crops are not treated every cropping season, as are annual crops, but only when the orchard or vineyard is replanted. Grapes had a value of \$2.8 billion in California in 2000, peaches a value of \$251 million, almonds a value of \$681 million and walnuts a value of \$289 million.[57] 'Replant disorder' is a general term for the lack of vigor in newly replanted orchards and vineyards as compared with trees and vines planted in 'non-replant' soil. Fumigation with methyl bromide prior to replanting alleviates this problem. Fumigation with 1,3-D has also been used to control replant disorder, but California's township limits for 1,3-D restrict the availability of this option in some areas.[58] Perennial crops have deep root systems, some of which remain in the soil after old trees or vines are removed. These deep roots can serve as a reservoir of pathogens and nematodes, ready to infect the new trees and vines as soon as they are planted. Field trials to evaluate potential alternatives for perennial crops must determine efficacy of control, not only at the time of planting but also the on-going performance during the early growth and fruiting years. Thus, each field trial requires several years and significant resources before sufficient data are obtained.

5.1 Overview

ARS research has investigated biological, chemical, cultural and genetic approaches to finding alternatives to methyl bromide for perennial crops. Apple replant disorder has been characterized as largely fungal in nature. A crop of wheat prior to replanting apple fostered a disease-suppressive microbial community.[59] Vines planted in fumigated soil responded to inoculation with vesicular–arbuscular (VA) mycorrhizae, suggesting that the beneficial organisms that are suppressed by some chemical treatments are necessary for vigorous vine growth and should be reintroduced following fumigation.[60] Chloropicrin, 1,3-D and 1,3-D + chloropicrin have resulted in tree and vine growth as good or better than with methyl bromide.[61–63] Shank-applied iodomethane gave good tree growth, but vine growth was intermediate between methyl bromide and untreated controls.[62,64] Recent trials indicate that shank- and drip-applied iodomethane and propargyl bromide and drip-applied chloropicrin, azide and 1,3-D + chloropicrin each controlled plant parasitic nematodes as well as methyl bromide at planting, but long-term efficacy is not yet known.[65] Fallowing for 3 years resulted in tree growth as good as or better than with methyl bromide for plum, but not for peaches.[63] A 3-year fallow in a grape replant trial resulted in a significant reduction in *Meloidogyne spp*, rootknot nematode, but not in *Tylenchulus semipenetrans* Cobb, citrus nematode. Tree and vine rootstocks can be resistant to a specific pest, but susceptible to others.[62,66] Accurate diagnosis of the problem in a specific orchard or vineyard will be critical to the selection of the best management option.

5.2 Research highlight: orchard replant

Replant disease of apple is typically controlled through the application of pre-plant soil fumigants, including methyl bromide, prior to orchard establishment on old orchard sites. Soil fumigation has been the option of choice due to the uncertain etiology of the disease. Systematic studies conducted in multiple orchards in Washington state utilized several approaches to define the causative agents of replant disease.[67] A fungal complex consisting of various species in the genera *Cylindrocarpon, Phytophthora, Pythium* and *Rhizoctonia* was shown to be the dominant cause of replant disease. Although implicated as a dominant causal agent in other geographical regions, the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Stekhoven, was shown to have a limited site-specific role in disease development in Washington, a finding consistent with previous reports.[68] Identification of the causal pathogen complex has enabled the formulation of biologically based management options for the control of apple replant disease.

Based on composition of the causal pathogen complex, several biological, cultural and narrow-spectrum chemical control options have been devised and are being evaluated. The application of selected independent treatments has, in certain instances, yielded promising results. Physical manipulation of the orchard environment through altering spatial arrangements of the orchard or soil disturbance, minimized the impact of replant disease and enhanced yield of Gala/M.26 at the Columbia View orchard. Soil disturbance entailed excavation to a depth of 0.5 m in the fall prior to planting and spreading the soil over the adjacent ground. This resulted in soil exposure to repeated freeze/thaw cycles, an event that can limit the survival and activity of certain soil-borne pathogens, including *Rhizoctonia solani* Kühn.[69] Trees were also established in the old orchard aisle, rather than tree row, which subjected newly established trees to an environment possessing a reduced disease potential. Both treatments enhanced growth and yield of Gala/M.26 at this site (Table 2). As the pathogen complex at this site was comprised of *Cylindrocarpon destructans* (Zinssm) Scholten, *Phytophthora cactorum* (Lebert & Cohn) Schroeter, *Pythium heterothallicum* Campbell & Hendrix and *Rhizoctonia solani* AG 6, but not *Pratylenchus penetrans*,[67] a soil drench consisting of the fungicides metalaxyl and flutolanil was employed. Although vegetative growth was initially suppressed by this fungicide treatment, disease control and yield at the second harvest were comparable to that obtained through methyl bromide fumigation (Table 2).

Table 2. Effect of cultural, biological and chemical methods on yield of apples cv 'Gala'/M26 planted on replant ground in 1998 at Columbia View orchard, Orondo, WA

Treatment	Year 2000 yields (kg per tree)[a]	Year 2001 yields (kg per tree)[a]
Untreated Control	4.6	20.64
Methyl bromide fumigation	7.2*	27.12*
Soil excavation	5.4	25.72*
Interplanting (aisle)	6.4*	—[b]
Pseudomonas putida 2C8	4.1	21.36
RootShield® (*T harzianum*)	4.7	22.45
Difenconazole	3.4	23.71
Metalaxyl+flutolanil	4.5	29.1*
Humic acid	3.4	19.9

[a] Means in a column followed by (*) are significantly different ($P = 0.05$) from the control.
[b] Trees removed from the aisle October 2000.

Table 3. Suppression of root infection by *Rhizoctonia solani* AG 5 and enhanced apple growth induced through wheat cultivation of WVC-Auvil orchard soil occurs in a cultivar specific manner

Treatment	Root wt (g)[ab]	Shoot wt (g)[ab]	% Root infection[ab]
Control	0.47 a	1.11 a	18.2 b
Pasteurized (95 C)[c]	0.89 bc	1.78 a	42.7 c
Hill-81	0.58 ab	2.59 b	16.7 b
Madsen	0.66 ab	2.42 b	18.9 b
Lewjain	1.04 c	4.79 c	3.3 a
Penawawa	0.94 bc	4.04 c	1.4 a

[a] After the wheat cultivation treatment, soil was infested with oat-bran inoculum[69,70] of *R solani* AG 5 isolate 5–103 at a rate of $1\,g\,kg^{-1}$.
[b] Means in the same column followed by the same letter are not significantly different ($P = 0.05$).
[c] Soils were cultivated to Lewjain wheat prior to pasteurization.

Induction of soil suppression of elements of the causal pathogen complex may have potential as a component in an integrated system for control of apple replant disease. In greenhouse trials, cultivation of multiple orchard replant soils with wheat induced soil suppression of an introduced isolate of *Rhizoctonia solani* AG 5. This pathogen incites Rhizoctonia root rot of apple[70] and contributes to replant disease development. Suppression was mediated through the resident soil microflora as evidenced by the elimination of disease suppression when soils were pasteurized following wheat cultivation but prior to introduction of *R solani* (Table 3). It is interesting that the induction of disease suppression was found to vary depending on the wheat genotype.[71] The wheat genotype-specific nature of the response was associated with specific transformations in the resident soil microbial community. One element of this transformation was the consistent selection by certain wheat genotypes of a fluorescent pseudomonad community that exhibited a significantly higher capacity to suppress *in vitro* growth of *R solani* AG 5 that was resident in the non-treated replant orchard soils (Table 4). Wheat varieties that did not induce disease suppression supported a fluorescent pseudomonad community that did not differ from the control in its ability to suppress fungal growth.

Table 4. *In vitro* suppression of *Rhizoctonia solani* AG 5 (zones of inhibition in mm) induced by fluorescent *Pseudomonas* spp obtained from the roots of Gala apple seedlings grown in WVC-Auvil orchard soils cultivated to wheat

Wheat cultivar	Average zone of inhibition (mm)[a]
No Wheat (Control)	0.71 a
Hill-81	0.91 ab
Lewjain	2.32 c
Madsen	0.60 a
Penawawa	1.65 bc

[a] Means in the same column followed by the same letter are not significantly ($P = 0.05$) different.

Based on results of these studies and associated field trials it is apparent that, with the development of appropriate guidelines, the integration of biologically sustainable management practices has significant potential as an alternative to pre-plant soil fumigation for control of apple replant disease.

5.3 Research highlight: vineyard replant

A 65-year-old 'Thompson Seedless' vineyard, located at the USDA Parlier, CA, research station was selected for a grape replant field trial. The treatments are described in Table 5. Each treatment was replicated five times in a randomized complete block design.

Table 5. Treatments applied to a vineyard replant field

Treatment 1	Untreated control
Treatment 2	1-Year fallow
Treatment 3	1-Year fallow plus a sorghum-sudangrass hybrid cover crop
Treatment 4	Shanked application of methyl bromide, 99.5% methyl bromide, 0.5% chloropicrin (448 kg ha^{-1}), tarped (the treated control)
Treatment 5	Shanked application of iodomethane (448 kg ha^{-1}), tarped
Treatment 6	Combination application of Telone II EC (327 litres ha^{-1} of 1,3-D) in 60 mm of water through a buried drip tape plus Vapam (243 litres ha^{-1} of 42% metam-sodium) through microsprinklers
Treatment 7	Combination application of Telone II EC (327 litres ha^{-1} of 1,3-D) in 60 mm of water through a buried drip tape plus Vapam (243 litres ha^{-1} of 42% metam-sodium) through microsprinklers + 1-year fallow
Treatment 8	Combination application of Telone II EC (327 litres ha^{-1} of 1,3-D) in 100 mm of water through a buried drip tape plus Vapam (243 litres ha^{-1} of 42% metam-sodium) through microsprinklers
Treatment 9	Combination application of Telone II EC (327 litres ha^{-1} of 1,3-D) in 100 mm of water through a buried drip tape plus Vapam (243 litres ha^{-1} of 42% metam-sodium) through microsprinklers +1-year fallow

Vines were removed from the 1-year fallow plots in fall, 1996. All other vines were removed in fall, 1997. 1,3-D + metam-sodium treatments were applied in early January, 1998. Methyl bromide and iodomethane treatments were applied in late April, 1998. In July of 1998, each plot was planted with three grape variety/rootstock combinations; own-rooted Thompson Seedless, Merlot on Harmony rootstock and Merlot on Teleki 5C rootstock. The rootstocks vary in levels of resistance to nematodes, which are thought to play a role in replant disorder. First- and second-year results of this study were reported previously.[72,73]

Soil samples were collected to a depth of 60 cm from each treatment/rootstock combination in June, 2001 and processed by sugar flotation–centrifugation.[74] Although seven different plant parasitic nematode genera occurred in the field, the predominant genera were the root-knot (*Meloidogyne spp*) and the citrus (*T semipenetrans*) nematode. Population levels for these two genera are given in Table 6. Three years after treatment, the 1,3-D + metam-sodium combinations and iodomethane continue to give reductions of population levels of the root-knot and citrus nematode comparable with that of methyl bromide. While still present, the reduction in root-knot nematode populations observed in previous years in the 1-year-fallow and 1-year-fallow + cover-crop treatments, is no longer statistically significant in Thompson seedless and Harmony plots. Untreated control and 1-year-fallow + cover-crop plots planted to the Harmony rootstock had significantly lower rootknot nematode populations than plots planted to Thompson seedless or Teleki 5C. Harmony supported the highest populations of citrus nematode and Teleki 5C the lowest. The difference was significant for both untreated and 1-year fallow plots.

Three years after treatment and re-planting, drip-applied 1,3-D and shank-applied iodomethane continue to give control of the root-knot and citrus nematode populations that is equivalent to that obtained with methyl bromide. The Harmony rootstock continues to support only minimal populations of the root-knot nematode, even in the untreated plots, but supports higher populations of the citrus nematode than either Thompson Seedless or Teleki 5C. Resistant rootstocks can be effective, but are more expensive than own-rooted vines and often not resistant to the diversity of pests that is encountered in a replant situation. If only root-knot nematode is present, the Harmony rootstock is a good alternative, alone or in combination with chemical controls, but citrus nematode populations will increase on Harmony if it is used without any chemical control. Iodomethane and the 1,3-D + metam-sodium combinations appear to be good alternatives to methyl bromide for vineyard replant when both root-knot and citrus nematode are present, at least for the first 3 years after establishment of a replanted vineyard.

Table 6. Nematode populations per 100 ml soil in soils receiving different treatments[abc]

	Meloidogyne sp			*Tylenchulus semipenetrans*		
Treatment	Thompson Seedless	Teleki 5C	Harmony	Thompson Seedless	Teleki 5C	Harmony
Untreated control	103.7 a	71.7 a	1.0 a	972.8 a	334.1 a	1216.0 a
1-Year fallow	85.8 a	27.7 b	0.0 a	661.1 a	291.8 a	1562.9 a
1-Year fallow plus cover crop	50.7 a	16.2 b	2.2 a	573.4 a	275.2 a	921.6 a
Methyl bromide (448 kg ha^{-1})	2.9 b	0.0 c	0.0 a	0.5 bc	2.2 b	108.2 b
Iodomethane (448 kg ha^{-1})	7.8 b	0.0 c	0.0 a	12.2 bc	3.0 b	58.7 b
Telone II EC (327 litres ha^{-1} of 1,3-D applied in 60 mm of water) + Vapam (243 litres ha^{-1})	0.2 b	0.0 c	4.5 a	1.1 bc	0.5 b	49.9 b
Telone II EC (327 litres ha^{-1} of 1,3-D applied in 60 mm of water) + Vapam (243 litres ha^{-1}) + 1-year fallow	0.2 b	0.0 c	0.0 a	0.0 c	0.0 b	5.6 b
Telone II EC (327 litres ha^{-1} of 1,3-D applied in 100 mm of water) + Vapam (243 litres ha^{-1})	33.9 b	0.0 c	0.0 a	37.4 b	15.4 b	4.5 b
Telone II EC (327 litres ha^{-1} of 1,3-D applied in 100 mm of water) + Vapam (243 litres ha^{-1}) + 1-year fallow	21.8 b	0.0 c	0.0 a	7.5 bc	1.1 b	59.5 b

[a] Mean of five replicates, soil sampled June 2001.

[b] Drip treatments applied January 1998; shank treatments applied April 1998.

[c] Means for each nematode genus/cultivar or nematode genus/rootstock combination followed by the same letter are not significantly different ($P = 0.05$).

5.4 Perennial field nursery crops

Soil fumigation with methyl bromide has commonly been used prior to planting field nurseries to insure that planting material grown for commercial and home-owner planting is free of soil-borne pathogens. Clean planting stock is critical not only for conventional growers, but also for organic growers. The California Code of Regulations makes it 'mandatory that nursery stock for farm planting be commercially clean with respect to economically important nematodes.'[75] The standard nursery treatment is methyl bromide. Fumigation with 1,3-D can be acceptable under some field conditions,[75] but its use is limited in California by township caps.[58] Growers of perennial nursery crops need an alternative to methyl bromide in order to continue to produce clean planting material and, if in California, to meet California Department of Food and Agriculture (CDFA) requirements. In order to be an acceptable management strategy, an alternative must be effective, available, economical and environmentally acceptable. In addition, to be approved as a nursery treatment in California, control of plant parasitic nematodes must be demonstrated to a soil depth of 152 cm (5 feet). In a grapevine nursery trial, shank-injected iodomethane and propargyl bromide and drip-applied chloropicrin, 1,3-D + chloropicrin, iodomethane, propargyl bromide and sodium azide were comparable with methyl bromide in the level of control of plant parasitic nematodes achieved to a depth of 152 cm.[65] Studies have been initiated recently to determine the impact of alternative fumigants and application technologies in garden rose and retail ornamental nurseries in California, but data are not yet available.

6 POST-HARVEST

Methyl bromide is used as a post-harvest fumigant to preserve product quality and to prevent the undesirable movement of insects, pathogens and nematodes that could be transported with commodities. Although quarantine use of methyl bromide is exempted under the Montreal Protocol, the loss of the larger pre-plant market for methyl bromide could result in reduced economic interest by manufacturers in continued production of this material. Loss of methyl bromide could mean the potential loss of several hundred million dollars in imports and exports of fruit, vegetables and nuts. ARS research programs have developed potential chemical and non-chemical replacement treatments for post-harvest use of methyl bromide.

6.1 Chemical alternatives

For fresh and durable commodities, time is often the most important factor in marketing the commodity. Consequently, replacements for methyl bromide can be categorized into those fumigants that require one day to several days and those which require only a few hours to be effective. Short treatment times are critical for fresh commodities because damage to the commodity is more likely to occur during long exposures. In additional, the large volumes of commodities to be treated often require quick turn-around time in the fumigation chamber. With this in mind, ARS scientists have concentrated on developing shorter time applications.

6.1.1 Iodomethane

Iodomethane is a logical choice to replace methyl bromide, but it is not registered for use on commodities. In trials using stored-product insects, it has been shown to be as active or more active than methyl bromide. In specific tests, iodomethane was effective in controlling pests such as Indianmeal moth, *Plodia interpunctella* (Hübner), navel orangeworm, *Amyelois transitella* (Walker) and codling moth, *Cydia pomonella* L.[76–80] One potential problem observed with iodomethane is its high rate of adsorption onto commodities.

6.1.2 Carbonyl sulfide

Carbonyl sulfide was developed in Australia as a grain fumigant and is undergoing registration there. It has good penetrating action and is toxic to a variety of stored product insects.[78] In disinfestation trials, carbonyl sulfide gave 100% mortality of diapausing codling moth larvae inside walnuts, when exposed for 24 h at a dosage of 40 mg litre^{-1}. The sorption of carbonyl sulfide on walnuts was less than that of either methyl bromide or iodomethane. Carbonyl sulfide is not an ideal quarantine fumigant because it requires lengthy treatment times to be effective against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).[81] One problem that was identified with carbonyl sulfide is that an odor often remains in the commodity following fumigation.[76] These objectionable odors may be caused by either wet commodity or contamination of the formulation with hydrogen sulfide.[82]

6.1.3 Sulfuryl fluoride

Sulfuryl fluoride (Profume®), used for many years as a structural fumigant under the trade name of Vikane®, has the advantage of being registered, but does not yet have food tolerances established. Studies have shown that sulfuryl fluoride is as active or more active against the adult, pupal and larval life stages, but less active against the egg stage, of insects than with methyl bromide.[76] Of the fumigants tested as alternatives, this compound exhibits the least sorption during fumigation and is an excellent penetrating fumigant.[76,83,84] When used on lemons and nectarines, sulfuryl fluoride caused phytotoxicity.[82]

6.1.4 Ozone

Ozone has the advantage of having a GRAS (Generally Considered As Safe) designation that exempts it from registration requirements. It has been shown to be effective against stored-product pests but it requires several hours when used alone and is a poor penetrant.[85] It is more effective when used with low levels of carbon dioxide, and vacuum may increase its toxicity or decrease the time required to obtain 100% mortality. It will require some special fumigation equipment because of its corrosive action.

6.2 Non-chemical methods

Efforts to develop non-chemical treatments for post-harvest use have concentrated on inert gases, heat/cold treatments, and radio frequency (RF) energy. In addition, some non-direct methods such as trapping and mating disruption of insects in the field might preclude conventional post-harvest treatments of codling moth and navel orangeworm.[86,87]

6.2.1 *Heat/cold treatments*

Hot air has proved a successful treatment for apple maggot, *Rhagoletis pomonella* Walsh.[88,89] Furthermore, it can eliminate fruit flies from Valencia oranges,[90,91] although some loss in flavor might occur due to loss in volatiles from the oranges.[92] Neven and Mitcham[93] demonstrated that combining controlled atmospheres with hot air greatly reduces the treatment time when compared with hot air alone. Low temperatures have been investigated for the elimination of Indianmeal moth and navel orangeworm from dried fruit,[94] but the long treatment times (days) suggest that these cold treatments would best be used as control disinfestations rather than as quarantine treatments.

6.2.2 *RF energy*

Results have shown that using long-wavelength energy to heat cherries in saline solution can be used as a quarantine treatment.[95] Also research shows that RF energy treatments are effective against codling moth and navel orange worm in walnuts.[96–98] At the wavelengths being studied, insects are selectively heated without adversely heating the fruit which might cause damage.

6.2.3 *Irradiation*

Irradiation has been shown to be the most efficient and least damaging method to treat some exposed fruit.[99,100] Generally, doses from 50 to 200 Gy are sufficient for quarantine security, but the exact dose varies with the insect being targeted. Four basic problems exist for using irradiation: (1) the capital costs for the facility are large and require that it operate year-round, (2) large volumes of commodities must be treated very quickly to allow timely movement through the marketing channels, (3) irradiation can render the adult stages sterile, rather than dead, leaving the inspector uncertain as to whether the insects were exposed to irradiation, whether all the insects were treated, or whether the adult entered the commodity following irradiation, and (4) many countries will not accept irradiation as a commodity treatment.

6.2.4 *Trapping/recycling of methyl bromide*

In 1995 to 1998, a cooperative agreement between ARS and industry investigated the most efficient method to capture methyl bromide on activated carbon. Studies showed that up to an 18% load of methyl bromide could be put on the activated carbon, depending upon temperature.[101] This research led to a commercial product and now two adsorption units are installed on working fumigation chambers in the USA.

7 CONCLUSION

The ARS research program on alternatives to pre-plant and post-harvest use of methyl bromide covers a diversity of cropping systems and fresh and durable commodities, utilizing a range of approaches including biological, genetic, cultural, chemical and physical control strategies. The program encompasses both short-term objectives to help growers and industry cope in the near term with the impending ban, and long-term objectives to transition to more integrated, sustainable management systems. Several short-term alternatives have been identified and evaluated for both pre-plant and post-harvest uses. Some will require regulatory approval before they will be available for commercial use. Good progress is being made in component research programs that will serve as the foundation for the development of integrated systems that begin with pre-plant preparation and end with commodity treatments.

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